Study to Assess Oropharyngeal Carriage of *N. Meningitidis* in South Australian School Leavers.

Clinicaltrials.gov: NCT03419533

Protocol V5, 7th November 2017

PHASE 2: AN OBSERVATIONAL CROSS SECTIONAL STUDY TO ASSESS NASOPHARYNGEAL CARRIAGE OF *N. MENINGITIDIS* IN SOUTH AUSTRALIAN UNIVERSITY STUDENTS AND SCHOOL LEAVERS

PHASE 2 (STUDY PART 2 AND STUDY PART 3) PROTOCOL SUMMARY

Title:

South Australian Men B vaccine herd immunity study.

Study Part 2: Pilot observational longitudinal study to assess nasopharyngeal carriage of *Neisseria meningitidis* in South Australian university students

Study Part 3: An observational cross sectional study to assess nasopharyngeal carriage of *Neisseria meningitidis* in South Australian school leavers.

Précis:

To estimate the prevalence of *N. meningitides* in first year university students and school leavers in South Australia posterior oro-pharyngeal swabs will be obtained prior to and post implementation of the school immunisation program (Phase 1). Potential risk factors will be assessed along with a comparison of carriage prevalence in vaccinated and unvaccinatedparticipants. Carriage will be estimated at baseline (2017) and in 2018, 2019 and potentially additionally in 2020 if funding available (post implementation of the school immunisation program (Phase 1).

Objectives:

Study Part 2: 2017 Pilot Longitudinal Carriage Study

Primary Objective

- Estimate the carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students.

Secondary Objectives

- Estimate the carriage prevalence of *N. meningitidis* genogroups causing disease *(A, B, C, W, X, Y)* in South Australian first year university students.
- Identify characteristics associated with carriage prevalence of all *N. meningitidis* genogroups in South Australian first year university students.
- Estimate the change in carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students at baseline (first week of university) and 3 months later Estimate any difference in PCR positivity after freezing of the sample at 6, 16 and 48 hours post collection.Estimate any difference in positivity of isolates determined by culture after freezing of samples at 6, 16 and 48 hours post collection

Study Part 3: 2018 /2019 /2020 Cross-sectional carriage study

Primary Objective

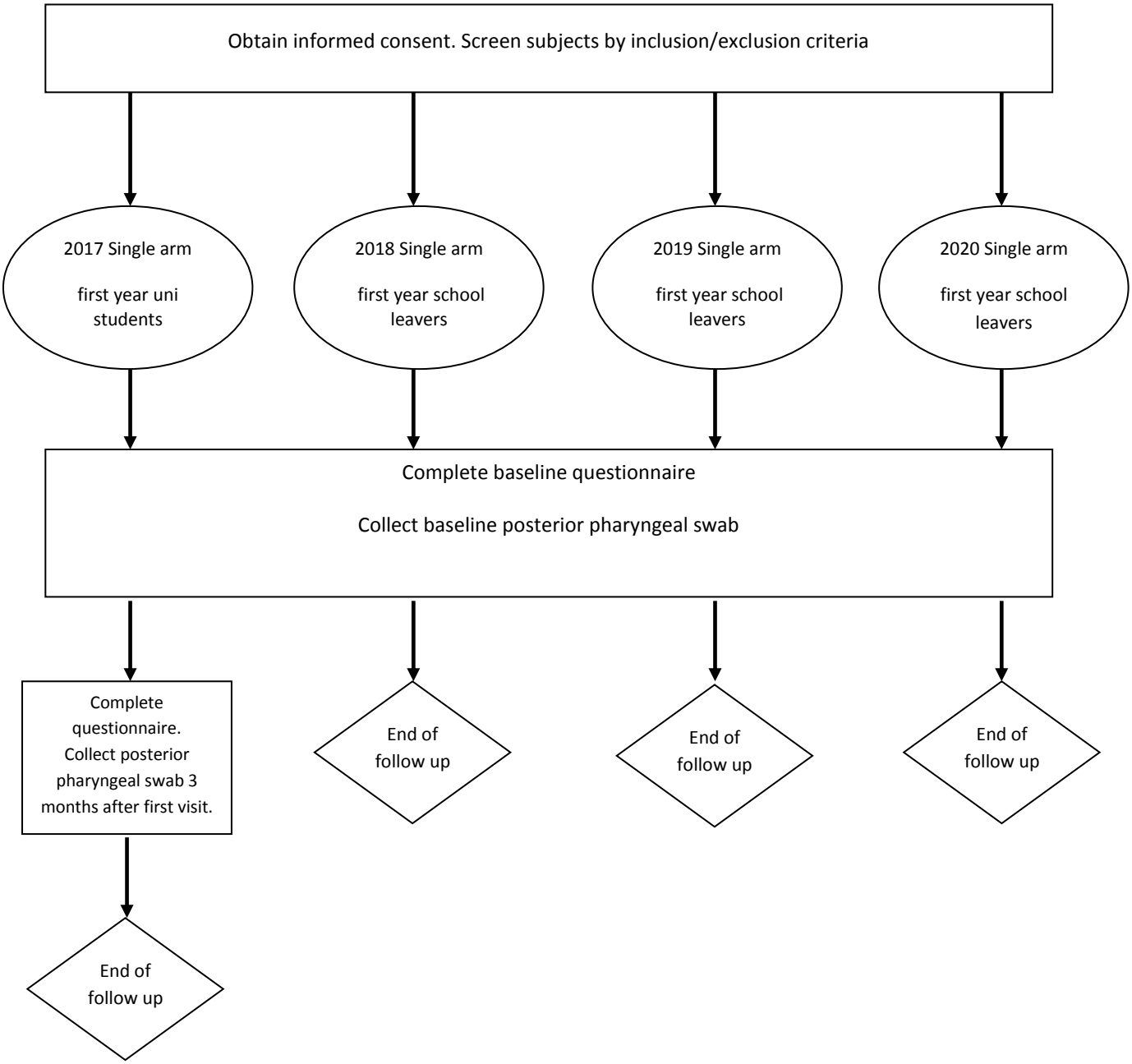
- Estimate the difference in carriage prevalence of disease causing genogroups of *N.meningitidis (A, B, C, W, X, Y)* in South Australian school leavers in 2018 (year 12 in 2017), 2019 (year 12 in 2018) and 2020 (year 12 in 2019)following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.

Secondary Objectives

- Estimate the difference in carriage prevalence of all N. *meningitidis* genogroups in South Australian school leavers in 2018 (year 12 in 2017),2019 (year 12 in 2018) and 2020 (year 12 in 2019) following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.
- Estimate the difference in carriage prevalence of each N. *meningitidis* genogroup *(A, B, C, W, X, Y)* in South Australian school leavers in 2018 (year 12 in 2017), 2019 (year 12 in 2018) and 2020 (year 12 in 2019) following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.
- Estimate the difference in carriage prevalence of all N. meningitidis genogroups in South Australian school leavers who have received Bexsero ® compared to unvaccinated students.Estimate the difference in carriage prevalence of N. meningitidis genogroups causing disease *(A, B, C, W, X, Y)* in South Australian school leavers who receivedBexsero ® , compared to unvaccinated students.
- Estimate the difference in each *N. meningitidis* genogroup carriage prevalence in South Australian school leavers who received Bexsero ®, compared to unvaccinated students.
- Identify characteristics associated with carriage prevalence of all *N. meningitidis genogroups* in South Australian school leavers in 2018 / 2019/ 2020.
- Identify characteristics associated with carriage prevalence of *N. meningitidis* genotypes causing disease *(A, B, C, W, X, Y)* in South Australian school leavers in 2018 /2019 /2020.

Primary Endpoint	The pilot study describing baseline carriage in South Australian university students will be completed in 2017. Carriage prevalence of *N. meningitidis* as determined by PCR
Population:	South Australian first year university students and school leavers.
Study Duration:	The study recruitment will commence in 2017 with independent cohorts recruited over a three year period.
Participant Duration:	Study Part 2: The pilot study has two time points with the total duration being about 3 months. Study Part 3: The participants have no ongoing involvement following a pharyngeal swab at visit 1.

STUDY PARTS 2 AND 3 SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

Study collaborating organisations

University of Adelaide, Adelaide SA (Sponsor)

Vaccinology and Immunology Research Trials Unit, Women's and Children's Hospital, Adelaide, SA (co-ordination & HREC)

SA Pathology, Adelaide, SA (Laboratory processes)

SA Health Communicable Disease Control Branch (co-ordination and IMD surveillance data)

SAHMRI – whole genome sequencing of N.meningitidis

Principal Investigator

Professor Helen Marshall, MBBS MD MPH DCH

Director, Vaccinology and Immunology Research Trials Unit (VIRTU)

Deputy Director, Robinson Research Institute, University of Adelaide

Discipline of Paediatrics

Women's and Children's Hospital

Email: *helen.marshall@adelaide.edu.au*

Co-Investigators

Associate Professor Ann Koehler BSc MBBS FRCPA MPH

Director, Communicable Disease Control Branch

System Performance & Service Delivery, SA Health

Associate Professor, School of Public Health, University of Adelaide

Email: *ann.koehler@sa.gov.au*

Mr Andrew Lawrence

Service Manager

Microbiology and Infectious Diseases

Royal Adelaide Hospital

SA Pathology

Email: *Andrew.Lawrence@sa.gov.au*

Dr Thomas Sullivan

Statistics Team Leader

Adelaide Health Technology Assessment

University of Adelaide

Email: Thomas.sullivan@adelaide.edu.au

Biostatistician and clinical trialist adviser

Emeritus Professor Philip Ryan

University of Adelaide

South Australia

Email: *philip.ryan@adelaide.edu.au*

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Adolescents are disproportionally affected by invasive meningococcal disease in SA. in 2016 in SA there were 27 IMD cases; 4 cases in children < 5 years, 3 in children 5-13 years, 12 cases in adolescents 14-24 years and 9 cases in adults 25-94 years of age. Over the last 10 years for adolescents aged 15 to 24 years, the highest number of reported IMD cases has been in 18 year olds (Figure 1). This age often coincides with finishing year 12 and commencing higher education, employment, or travelling. Nasopharyngeal carriage of *N. meningitidis* also has been shown to peak around 18-19 years of age at 24% with a sharp rise in carriage rates from 15 years of age (Figure 2).(5)

Figure 1: Invasive Meningococcal disease cases in South Australia since 2005 in 15 to 24 year olds.

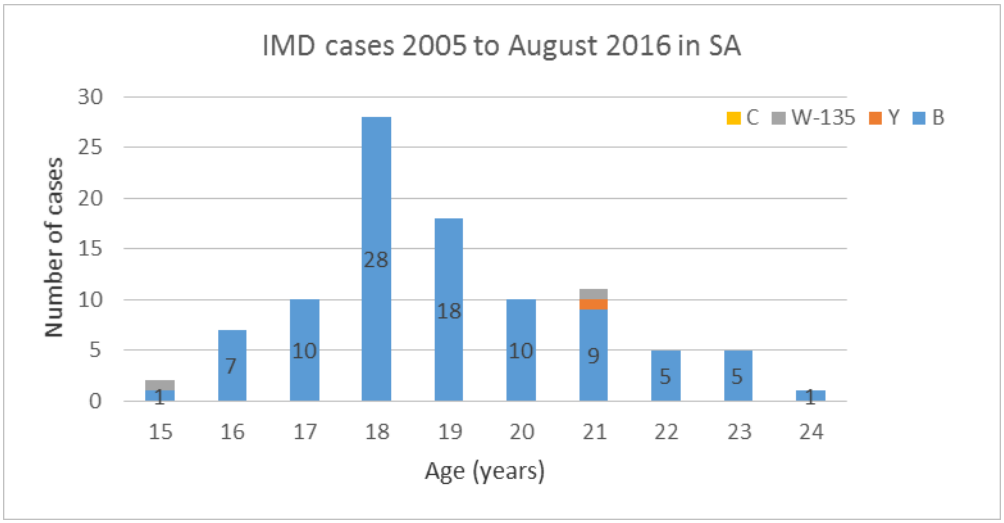
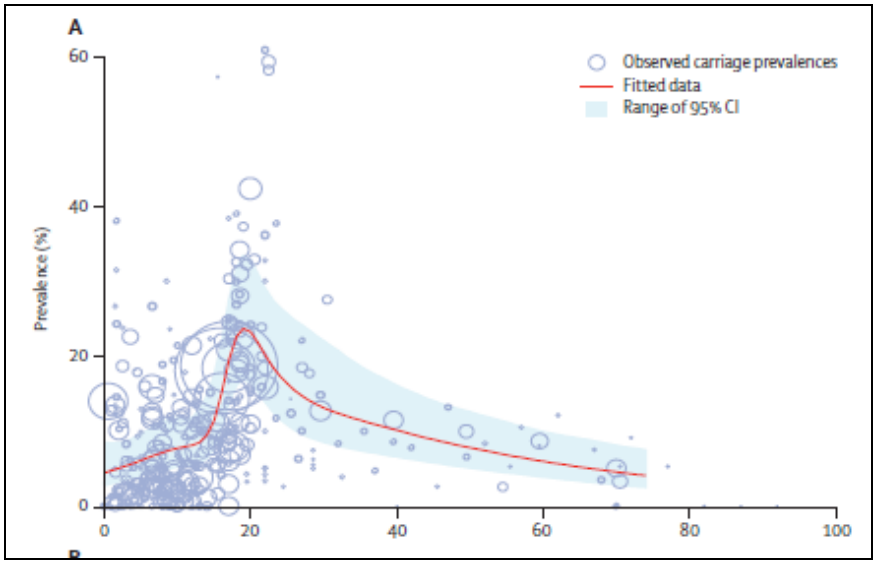


Figure 2: Meningococcal carriage by age (Systematic review, Christensen et al, Lancet Infectious Diseases)(5)



Pilot Study Part 2:Longitudinal carriage study

Data in relation to carriage in Australia are scant and very little is known about overall carriage prevalence of *Neisseria meningitidis* (i.e. all strains), or the proportion of strains that are meningococcal serogroup B. The only Australian data available in the literature reporting 1.7% carriage were from a sample of 294 school aged children of various ages in Queensland and was conducted in 1989.(12) The improvement in sensitivity of newer molecular diagnostic techniques and the fact that South Australia regularly reports higher Invasive Meningococcal Disease rates than Queensland leads us to suspect that baseline carriage in South Australian adolescents will be higher than 1.7%. In carriage studies conducted in Europe, carriage prevalence was estimated at 4·5% in infants to a peak of 23·7% in 19-year olds. Recently presented carriage rates from Nottingham University in the UK ranged from 14.3% - 46.2% for all serogroups at the commencement of the university year and 6 months later.[Oldfield N et al. IPNC Mancester, UK, September 2016] Carriage rates for serogroup B ranged from 3.3% to 8.5% over the same time period. As no carriage data exist in Australia since commencement of the meningococcal C vaccine program, we will conduct a longitudinal pilot study of 500 first year university students during orientation week in 2017 at the University of Adelaide (Study Part 2). These students will undergo a posterior pharyngeal swab and complete a short questionnaire to collect information about possible risk factors at the beginning of university and then 3 months later.

Age is one the most important factors influencing carriage, with peak carriage occurring around 18- 19 years of age. Other factors that influence carriage are being male, concomitant respiratory infections, active and passive smoking, number and closeness of social contacts, and low socioeconomic status.(6) Disease is a rare outcome of infection (5) but the relationship between carriage and disease incidence is not fully understood. With carriage rates significantly higher in adolescents, a reduction in carriage in this group has the potential to provide protection to unvaccinated people (herd immunity), including infants.

Study Part 3: Cross-sectional carriage study

A carriage study of a cohort of school leavers in SA will provide us with an opportunity to assess carriage rates in the population following introduction of an intervention in younger age groups (year 10, 11, 12) and compare carriage in individuals who have previously been vaccinated (in years 11 and 12) with individuals who are not vaccinated or did not wish to be vaccinated as part of Phase one. We plan to conduct a cross sectional carriage study of school leavers (including first year university students) who are at the age group at highest risk of invasive meningococcal disease in SA. We will also assess any association between various known risk factors and past history of 4CMenB vaccine on prevalence of nasopharyngeal carriage.

2.2 RATIONALE

Study Part 2: This pilot study will establish baseline carriage prevalence in 500 first year university students. Very few students will have previously been vaccinated with a MenB vaccine as this study will be conducted prior to Phase 1. The findings will assist in validation of study processes for Phase 1, particularly laboratory processes. A follow up swab taken 3 months later will provide data on the change in carriage that may occur over this 3 month period. Study Part 3 will evaluate carriage rates following introduction of a meningococcal B vaccine program, compare carriage rates in vaccinated and unvaccinated individuals and determine risk factors for carriage prevalence of *N. meningitidis* genogroups causing disease in South Australian school leavers. School leavers are defined as students who were enrolled in year 12 at school in the preceding year of recruitment and have been chosen as they are likely to have the highest carriage rates. Following completion of Phase 1, a high proportion (approximately two thirds) of students will be immunised by the time of throat swab collection in years 2018 and 2019. Assessing carriage in both vaccinated and unvaccinated cohorts will further inform the effect the 4CMenB vaccine has on carriage at a population level. This will help inform national immunisation program funding of 4CMenB vaccine in adolescents in Australia and internationally.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

This is an observational study with minimal risks to the participants. Oral pharyngeal swabs may be uncomfortable, and may cause a gagging sensation however there are unlikely to be any other effects associated with this procedure.

2.3.2 KNOWN POTENTIAL BENEFITS

There will be no direct benefits from this study for the participants. Information from this study will assist in determining the potential herd immunity effects of meningococcal B vaccines. These data are important to inform the cost effectiveness of national and international funded MenB vaccine programs in adolescents.

3 OBJECTIVES AND PURPOSE

Pilot Study Part 2: Longitudinal Carriage Study

Primary Objective

- Estimate the carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students.

Secondary Objectives

- Estimate the carriage prevalence of *N. meningitidis* genogroups causing disease *(A, B, C, W, X, Y)* in South Australian first year university students.
- Identify characteristics associated with carriage prevalence of all *N. meningitidis* genogroups in South Australian first year university students.
- Estimate the change in carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students at baseline (first week of university) and 3 months later.
- Estimate any difference in PCR positivity after freezing of the sample at 6, 16 and 48 hours post collection.
- Estimate any difference in positivity of isolates determined by culture after freezing of samples at 6, 16 and 48 hours post collection

Study Part 3: Cross-sectional Carriage Study

Primary Objective

- Estimate the difference in carriage prevalence of disease causing genogroups of *N.meningitidis (A, B, C, W, X, Y)* in South Australian school leavers in 2018 (year 12 in 2017),2019 (year 12 in 2018) and 2020 (year 12 in 2019)following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.

Secondary Objectives

- Estimate the difference in carriage prevalence of all N. *meningitidis* genogroups in South Australian school leavers in 2018 (year 12 in 2017), 2019 (year 12 in 2018) and 2020 (year 12 in 2019) following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.

- Estimate the difference in carriage prevalence of each N. *meningitidis* genogroup *(A, B, C, W, X, Y)* in South Australian school leavers in 2018 (year 12 in 2017), 2019 (year 12 in 2018) and 2020 (year 12 in 2019) following implementation of a school immunisation program in year 10, 11 and 12 South Australian students from 2017-2018.
- Estimate the difference in carriage prevalence of all N. meningitidis genogroups in South Australian school leavers who have received Bexsero ® compared to unvaccinated students.
- Estimate the difference in carriage prevalence of N. meningitidis genogroups causing disease *(A, B, C, W, X, Y)* in South Australian school leavers who receivedBexsero ® , compared to unvaccinated students.
- Estimate the difference in each *N. meningitidis* genogroup *(A, B, C, W, X, Y)* carriage prevalence in South Australian school leavers who received Bexsero ®, compared to unvaccinated students.

- Identify characteristics associated with carriage prevalence of all *N. meningitidis genogroups* in South Australian school leavers in 2018 / 2019/ 2020

Identify characteristics associated with carriage prevalence of *N. meningitidis* genotypes causing disease *(A, B, C, W, X, Y)* in South Australian school leavers in 2018 /2019 /2020.

Exploratory objectives

- Describe *N. meningitidis* carriage density for all genogroups in school leavers using qPCR
- Describe genome sequencing of pathogenic *N. meningitis* (A, B, C, W, X, Y) in school leavers.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

Study Part 2: An observational longitudinal study to estimate the carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students. This study recruitment will commence during orientation week (20th to 24th of February 2017). Students that are enrolled will have a follow up swab taken 3 months later.

The student association and university will circulate information about the study to students. This will include website coverage and flyers in the orientation week student information packs. A stall will be set up within Adelaide University to enroll university students into the study. The second swab will be taken to coincide with timing of vaccination and swabs in the school immunisation program. A private screened section will be provided to conduct the throat swabs. A questionnaire will be completed at each visit.

Study Part 3: An observational cross sectional study to evaluate the impact of 4CMenB vaccination and known risk factors on carriage in school leavers. Students previously enrolled in the MenB vaccine herd immunity study will also be contacted and offered participation in part 3 of the study to provide an additional swab as they become school leavers. The University of Adelaide, University of South Australia, and Flinders University will be approached to participate with recruitment of students at each university during orientation week and for several weeks after until recruitment has been completed. University health services and involved general practices and council immunisation providers may also be involved in collection of swabs for study part 3 . Similar to Study Part 2, we will work with the universities and student organisations to disseminate information about the study.

4.2 STUDY ENDPOINTS

Primary Endpoints

Study Part 2:

Carriage prevalence of all *N. meningitidis* genogroups as measured by PCR at baseline and 3 months later in first year university students.

Study Part 3:

Carriage prevalence of *N. meningitidis* genogroups as measured by PCR in school leavers

Secondary Endpoints

Study Part 3:

Carriage prevalence of all *N. meningitidis* genogroups as measured by PCR in vaccinated and unvaccinated school leavers

Exploratory Endpoints

Study Part 3:

1. Carriage density of *N. meningitidis* genogroups as measured by qPCR in school leavers in 2018 and 2019.

2. Whole genome sequencing of invasive isolates (genogroup A, B, C, W, X, Y)

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

Individuals eligible to be enrolled into this study are:

- South Australian school leavers between 17 to 25 years of age (enrolled in year 12 in the previous year ie year 2018, students are eligible if they were enrollled in year 12 in 2017, year 2019, students are eligible if they were enrolled in year 12 in 2018 and year 2020, students are eligible if they were enrolled in year 12 in 2019).

5.2 PARTICIPANT EXCLUSION CRITERIA

- No exclusion criteria

5.3 STRATEGIES FOR RECRUITMENT

Study Part 2: In 2017, university students from The University of Adelaide will be approached to be involved in the study. The sampling frame will occur during orientation week and 500 students will be recruited to provide posterior oro-pharyngeal swabs. Students will be provided with a $20 iTunes voucher to reimburse their time for each visit.

Study Part 3: Adelaide's three largest universities (The University of Adelaide, University of South Australia, and Flinders University) will be approached to be involved in the study in 2018 and 2019 and potentially 2020. All school leavers aged between 18-25 years will be invited to enrol in the study by use of advertising in the university and distribution of postcards and through council immunisation clinics in 2018. Year 12 students in Group B who will be returning for 2 doses of Bexsero will be provided with a letter and SMS text message reminding them of their vaccinations that are due and the opportunity to further participate with an additional throat swab. At the end of each year a letter will be provided to schools to distribute to all students in year 12 to invite their participation for a throat swab the following year. An Enrolled Nurse, Registered Nurse, or Medical Officer will collect a posterior pharyngeal swab from all enrolled students. We will be aiming to recruit 4096school leavers from February – June each year.. The student will be required to fill out a questionnaire and provide a one off posterior pharyngeal swab. The survey will collect information on potential risk factors (prior vaccination, smoking history, household size, recent antibiotic use) for the carriage of N. meningitidis. No ongoing visits are required. Participants will be offered a $40 voucher to compensate them for travel expenses.

A communications officer will be employed to work with university students union and public relations to advertise the study to university students at all 3 universities on establishing appropriate and accessible avenues of communication. Involving students in the planning and delivery of communication strategies is expected to facilitate two way communication and provide opportunities for students to engage in research.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Participants may withdraw from the study at any time at their request.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

As per Phase 1

6 STUDY PROCEDURES AND SCHEDULE

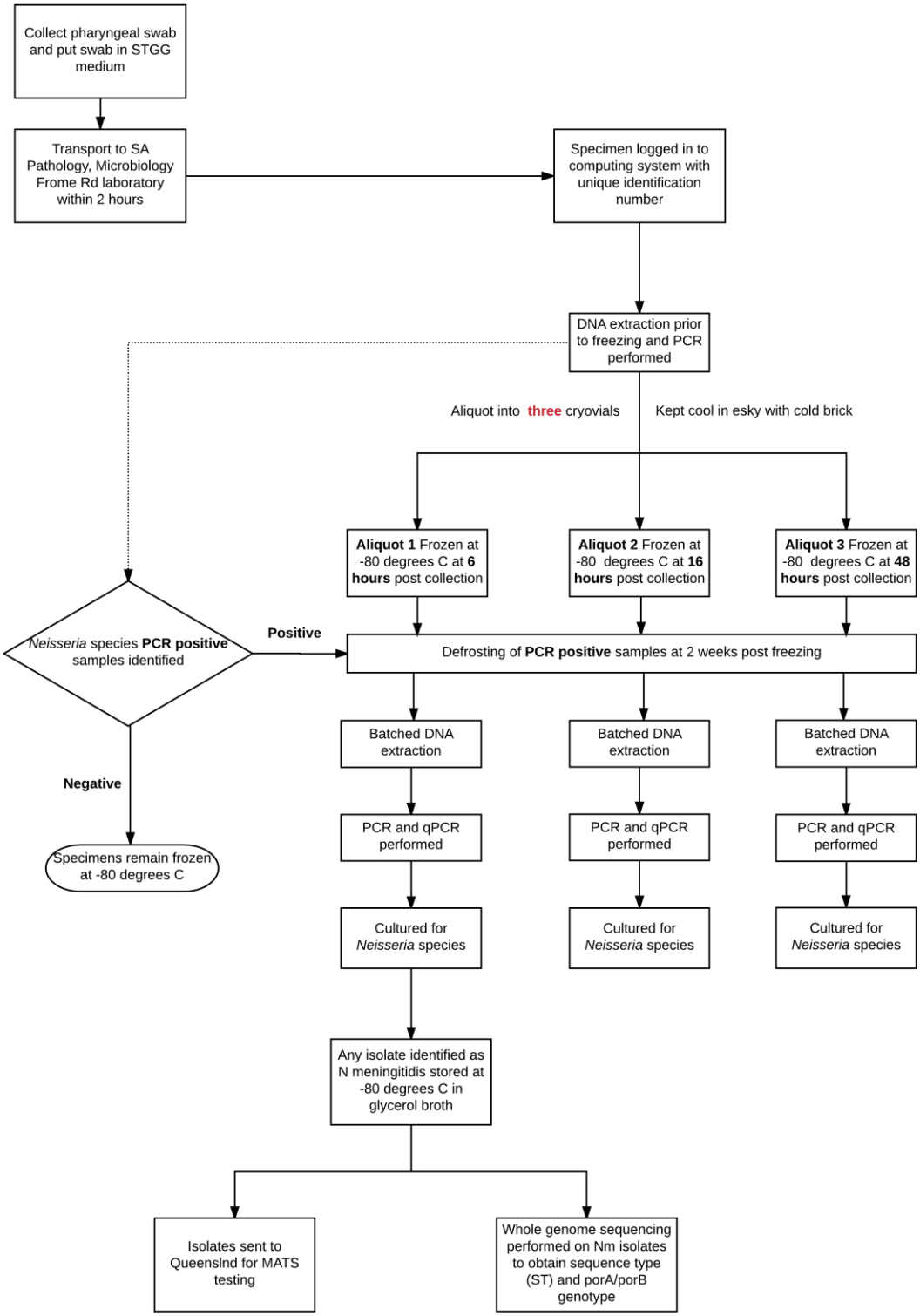
6.1 STUDY PROCEDURES/EVALUATIONS

6.1.1 STUDY SPECIFIC PROCEDURES

Pharyngeal swabs will be collected from participants using a standard operating procedure (SOP) to ensure consistent and optimal collection and handling of swabs. A swab will be placed at the back of the throat and swept across the posterior pharynx and withdrawn. Laboratory procedures/evaluations are described in Phase 1.

Study part 2 will additionally measure any difference in PCR positivity for *N. meningitidis* following freezing at 6 hours post collection and 16 and 48 hours post collection. This will assist in validating laboratory procedures for the RCT study. Once the sample has been received by SA Pathology each sample will be aliquoted into three cryovials and frozen at three different timepoints (6 hours, 16 hours, and 48 hours) following collection time.

Specimen and laboratory flow diagram for study part 2



6.2 STUDY SCHEDULE

6.2.1 COMMUNICATION

A participant information sheet and consent form will be provided to potential participants describing the intent of the study, what is involved, potential risks, potential benefits, and consent process.

6.2.2 ENROLMENT

Visit 1 – Month 0, Day 0 (Study Part 2 and study Part 3)

- Study staff (EN, RN, or MO) will provide each participant with an information sheet and consent form.
- They will obtain written informed consent from each participant.
- A questionnaire will be provided that will ask for the following information:
    - First name
    - Surname
    - Gender
    - Date of birth
    - Year of schooling
    - Name of school
    - Attendance at university or occupation
    - Enrolment in the B Part of it study
    - Medicare number
    - Prior receipt of 4CMenB vaccine (verification of previous Bexsero ® administration where possible)
    - Year and month of 4CMenB vaccination
    - Cigarettes smoked/day
    - Other smokers at home
    - History of meningococcal disease
    - Number of pub and club visits in the last week
    - Number of kissing contacts
    - Number of persons sharing a bedroom
    - Recent antibiotic use (none, current, stopped last week, stopped last month)
    - Number of people currently residing in their household
- Conduct pharyngeal swab collection
- Provide voucher to reimburse travel costs

Visit 2 – Month 3, Day 0 (Study Part 2 only)

- A questionnaire will be provided as per visit 1
- Conduct pharyngeal swab collection
- Provide iTunes card

6.3 LABORATORY PROCEDURES/EVALUATIONS

6.3.1 CLINICAL LABORATORY EVALUATIONS

Pharyngeal swabs will be collected from all students initially at baseline. A standard operating procedure (SOP) will be used to ensure consistent and optimal collection and handling of swabs. Samples will be evaluated for the presence of specific meningococcal DNA and if necessary also be used as target for molecular typing assays such as multi-locus sequence typing (MLST) and whole genome sequencing (WGS). Any sample with a positive

molecular assay will then be cultured using classical bacteriology methodology in order to isolate the *Neisseria* species. These isolates can then be characterised by molecular or other means. All laboratory techniques used for molecular testing and further typing are currently in use in the SA Pathology Microbiology and Infectious Diseases Laboratories. These laboratories are accredited by NATA/RCPA for routine testing of human and biological samples. All protocols are documented and reviewed regularly. They have been subject to appropriate validation according to NATA/NPAAC guidelines. Any new molecular assay e.g. detection of *sodC* gene of *N. meningitidis* will need to be internally validated before routine use.

6.3.2 OTHER ASSAYS OR PROCEDURES

All specimens will be subjected to PCR screening for the presence of specific meningococcal DNA (using *PorA* or *sodC* gene detection). Further molecular analysis will be used to determine which serogroup has been detected (A, B, C, W, X and Y). A range of other molecular tests can be performed on isolates of *Neisseria* species in order to subtype them. This includes serogroup determination, MLST typing, porA and porB typing whole genome sequencing and metagenomics. For isolates identified as *N. meningitidis,* further typing as described above will be performed and isolates stored in glycerol broth at -80 degrees Celsius (^{o}C) for further analysis.

A number of samples, particularly strains known to be associated with invasive disease will also be subjected to quantitative polymerase chain reaction (qPCR) in order to determine the density of carriage of meningococci. Appropriate well defined calibrated and validated controls will be used as comparators to determine the density of the carriage.

6.3.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

A 2mL skim milk-tryptone-glucose-glycerol (STGG) swab will be used for both molecular and classical bacteriological culture methodologies. A request form with details of the swab will accompany each sample. Once the samples reach the laboratory, details will be entered into the Laboratory Information System for recording, workup and subsequent reporting. The original samples can be stored at -80^{o}C until processed. Once processing is complete, the samples will be retained at -80^{o}C. All specimens will be subjected to DNA extraction using the Roche MagNaPure extraction platform for subsequent molecular testing using routine and standard protocols. The remaining fluid will be restored at -80^{o}C in case of a need for further analysis.

6.3.4 SPECIMEN SHIPMENT

The STGG swabs are the collection swabs and transport material of choice, since they are suitable for both molecular and bacterial culture assays. Once collected, they can be kept cool in styrofoam containers(eskies) and transported to the nearest SA Pathology collection centre or delivered to the Frome Road or nRAH laboratories directly. Transport of swabs from SA Pathology Collection centres to the central laboratory will be kept cool using validated transport methods for biological specimens.

7 ASSESSMENT OF SAFETY

7.1 REPORTING PROCEDURES

7.1.1 ADVERSE EVENT REPORTING

Adverse events and Serious Adverse events are not expected from Study Parts 2 & 3. Although we do not expect any SAE's to occur in relation to this study, any that occur will be immediately reported to the Principal Investigators and to the relevant Human Research Ethics Committee. In particular, if the study related SAE is fatal or life threatening, notification to the Principal Investigator and vaccine manufacturer must be made immediately, irrespective of the extent of available event information. Only SAEs that are assessed as related to study participation will be reported to the REC.

Reporting period

For each participant, the study related serious adverse event reporting period beings at the time of the participant's informed consent, and continues until study participation is complete. If the study investigators become aware of a study related serious adverse event after study completion this will also be reported.

Causality assessment

The investigator is required to assess and record the causal relationship. For all SAEs, sufficient information should be obtained by the investigator to determine the causality of each SAE. The investigator is required to follow-up the SAE until the event and/or its sequelae resolve or stabilize at a level acceptable to the investigator.

An investigator's causality assessment is the determination of whether there exists a reasonable possibility that any study processes caused or contributed to an adverse event.

- Specific Serious Adverse Events (SAE) reporting requirements are not needed in this as this study is non-interventional
- Any SAE assessed as related to study participation will be reported to the REC.
- The investigators will provide or arrange for prompt diagnosis and medical treatment of any research related injury experienced by a study subject. Investigators will also notify the vaccine manufacturer of any research related injuries from Study Parts 2 or 3.

7.2 SAFETY OVERSIGHT

Safety oversight will be under the direction of the PI, as for study 1.

8 CLINICAL MONITORING

As per Phase 1

9 STATISTICAL CONSIDERATIONS

9.1 DESCRIPTION OF STATISTICAL METHODS

9.1.1 GENERAL APPROACH

Logistic regression with adjustment for confouding will be used to identify factors associated with carriage and to compare carriage rates annually and between vaccinated and unvaccinated students. Changes in carriage between baseline and 3 months later will be evaluated using logistic regression with generalised estimating equations. In all analyses of carriage prevalence, effects will be described using odds ratios with 95% confidence intervals.

For comparing the difference in PCR +ve samples after freezing of the sample at 6 hours and 48 hours, we will compare paired proportions using a logistic model with generalised estimating equations, and assess agreement using a Kappa statistic.

9.1.2 BASELINE DESCRIPTIVE STATISTICS

Descriptive statistics will report percentages, means with standard deviations, median, and/or range (as appropriate) for the following:

- Age
- Socio-economic Index for Areas (SEIFA) categories. Calculated from suburb data
- Categorical outcomes for household size
- Categorical outcomes for number people that smoke in each house
- Prevalence of *N. meningitidis* all genogroups carriage
- Prevalence of *N. meningitidis* serogroup B carriage

9.2 SAMPLE SIZE

Study Part 2:. Assuming a carriage prevalence of approximately 8%, a sample size of 500 university students will allow for estimation of carriage prevalence with a precision of +/- 2.4%, where precision is defined as the half-width of a 95% confidence interval.

Study Part 3:. Assuming the carriage prevalence of disease causing genogroups of *N.meningitidis (A, B, C, W, X, Y)* in school leavers in 2018 is 8%, a sample size of 4,096 students per year will provide 80% power to detect a 20% relative reduction in carriage prevalence between 2018 and 2019 (two tailed alpha = 0.05).

10 QUALITY ASSURANCE AND QUALITY CONTROL

As per Phase 1.

11 ETHICS/PROTECTION OF HUMAN SUBJECTS

11.1 ETHICAL STANDARD

As per Phase 1

11.2 INSTITUTIONAL REVIEW BOARD

As per Phase 1

11.3 INFORMED CONSENT PROCESS

11.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing study procedures, and risks will be given to the participant and written documentation of informed consent is required prior to commencing any study procedures.

11.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be HREC approved and the participant will be asked to read and review the document. The EN, RN, MO will answer any questions that may arise. All participants will receive a written explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions by speaking with the study staff. The participant will sign the informed consent document prior to any procedures being done for the study. The participants may withdraw consent at any time throughout the course of the study. The participant will be provided with an copy of the information sheet.

11.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators and SA Pathology. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party without prior written approval of the sponsor.

Study investigators will store identifiable information in locked offices and compactors in the Vaccinology Immunology Research Trials Unit (VIRTU) at the Women's and Children's Hospital. Electronic data including student name, address, and telephone number will be stored on a protected sever at the University of Adelaide.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Women's and Children's Hospital. This will not include the participant's contact or identifying information. The study data entry and study management systems used by VIRTU research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the University of Adelaide.

This study is not a human genome-wide association study and no human genetic material will be analysed.

11.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

- Intended use: samples and data collected under this protocol may be used to investigate *N.meningiditis* carriage, density, serotype, genotype, and antigen type. No genetic testing will be performed on human material.

- Storage: samples and data will be stored using codes assigned by SA Pathology or the study team. Data will be kept in password-protected computers. Only investigators, study staff, and SA Pathology staff will have access to the samples and data. Patient identification data will be entered into the secure laboratory information system. Consolidated reports can then be generated from this system. All staff who have access to the laboratory information system are bound by the Public Sector rules and regulations pertaining to patient and result confidentiality.

- Tracking: Data will be tracked using accredited SA Pathology procedures and systems.
    - Disposition at the completion of the study: All stored samples will remain in frozen storage at SA Pathology and may be used for further testing to assess the N. meningititidis and associated microbiota.

11.5 FUTURE USE OF STORED SPECIMENS

After the study is completed, biological samples will be stored at SA Pathology and/or University of Adelaide. These samples could be used for research into *N.meningiditis* genotype and vaccine antigen coverage. Samples will be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant if they are to be sent to external laboratories.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

12 DATA HANDLING AND RECORD KEEPING

12.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the EN, RN, MO, and trial staff at VIRTU. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Clinical laboratory data will be entered into existing SA Pathology data systems. These will then be exported and combined in a database on the University of Adelaide Server (AHTA). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Once matched and the data is cleaned it will be de-identified. The Investigator is responsible for keeping a code list so that it is possible to link a participant code to a particular participant. This code list will be kept separately on the University of Adelaide server to ensure that in the case of an emergency, a participant can be identified.

12.2 STUDY RECORDS RETENTION

As per Phase 1

13 STUDY ADMINISTRATION

13.1 STUDY LEADERSHIP

As per Phase 1

14 LITERATURE REFERENCES

1. Chang Q, Tzeng Y, Stephens D. Meningococcal disease: changes in epidemiology and prevention. Clin Epidemiol. 2012;4:237-45. Epub 2012/10/17. doi: 10.2147/clep.s28410. PubMed PMID: 23071402; PubMed Central PMCID: PMCPMC3470458.

2. Jafri RZ, Ali A, Messonnier NE, Tevi-Benissan C, Durrheim D, Eskola J, et al. Global epidemiology of invasive meningococcal disease. Popul Health Metr. 2013;11(1):17. Epub 2013/09/11. doi: 10.1186/1478-7954-11-17. PubMed PMID: 24016339; PubMed Central PMCID: PMCPMC3848799.

3. Lahra M, Enriquez R. Australian Meningococcal Surveillance Programme annual report, 2014: Australian Government Department of Health; 2016 [cited 2016 16th August]. Available from: *http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-annlrpt-menganrep.htm*.

4. Invasive meningococcal disease national surveillance report. Australian Government Deparment of Health, 2016.

5. Christensen H, May M, Bowen L, Hickman M, Trotter C. Meningococcal carriage by age: a systematic review and meta-analysis. The Lancet Infectious diseases. 2010;10(12):853-61. Epub 2010/11/16. doi: 10.1016/S1473-3099(10)70251-6. PubMed PMID: 21075057.

6. Caugant D, Maiden M. Meningococcal carriage and disease--population biology and evolution. Vaccine. 2009;27 Suppl 2:B64-70. Epub 2009/05/26. doi: 10.1016/j.vaccine.2009.04.061. PubMed PMID: 19464092; PubMed Central PMCID: PMCPMC2719693.

7. Invasive meningococcal disease (IMD) [Internet]. European Center for Disease Prevention and Control. 2012 [cited 18 July 2016].

8. Maiden MC, Ibarz-Pavon AB, Urwin R, Gray SJ, Andrews NJ, Clarke SC, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. J Infect Dis. 2008;197(5):737-43. Epub 2008/02/15. doi: 10.1086/527401. PubMed PMID: 18271745.

9. Trotter CL, Maiden MC. Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. Expert Rev Vaccines. 2009;8(7):851-61. Epub 2009/06/23. doi: 10.1586/erv.09.48. PubMed PMID: 19538112; PubMed Central PMCID: PMCPMC3988355.

10. Australian Technical Advisory Group on Immunisation (ATAGI) Statement. Advice for immunisation providers regarding the use of Bexsero ® Immunise Australia Program: Australian Government; 2015 [cited 2016 16th August]. Available from: *http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/atagi-advice-bexsero*.

11. Read R, Heath P, et al. A phase 3 observer blind randomized, multi-center, controlled study to evaluate the effect of Novartis Vaccine's Meningococcal B recombinant and MenACWY conjugate vaccines on pharyngeal carriage of N meningitidis in young adults. Interim report. 2014.

12. Ingram SB, Wilson BJ, Kemp RJ, Faoagali J. Neisseria meningitidis in a school population in Queensland. The Medical journal of Australia. 1990;152(6):332. Epub 1990/03/19. PubMed PMID: 2314341.

13. MacLennan J, M. M. UKMENCAR4 meningococcal carriage study. International Pathogenic Neisseria Conference (IPNC). Manchester, England: 2016.

14. National notification disease surveillance system (NNDSS) [Internet]. Commonwealth Government. 2017 [cited 21st February 2017]. Available from: *http://www9.health.gov.au/cda/source/rpt_4.cfm*.

15. Lahra MM, Enriquez RP. Australian Meningococcal Surveillance Programme annual report, 2012. Communicable diseases intelligence quarterly report. 2013;37(3):E224-32. Epub 2013/01/01. PubMed PMID: 24890958.

16. Information and services exclusively for US Healthcare professionals: GSK; 2016 [cited 2016 16th August]. Available from: https://*http://www.gsksource.com/pharma/content/gsk/source/us/en/brands/bexsero/pi/po.html*.

17. Bexsero meningococcal B vaccine Australian Government Department of Health Australian Government; 2016 [cited 2016 16th August]. Available from: https://*http://www.tga.gov.au/monitoring-communication/bexsero-meningococcal-b-vaccine*.

18. Langley J, D. M, Halperin B, Swain A, Halperin S, Top K, et al. Rapid surveillance for health events following a mass meningococcal B vaccine program in a university setting: A Canadian Immunization Research Network study. Vaccine. 2016;34(34):4046-9. doi: *http://dx.doi.org/10.1016/j.vaccine.2016.06.025*.

19. Esposito S, Zampiero A, Terranova L, Montinaro V, Peves Rios W, Scala A, et al. Comparison of posterior pharyngeal wall and nasopharyngeal swabbing as a means of detecting the carriage of Neisseria meningitidis in adolescents. Eur J Clin Microbiol Infect Dis. 2013;32(9):1129-33. Epub 2013/03/19. doi: 10.1007/s10096-013-1856-2. PubMed PMID: 23504236.

20. The Australian Immunisation Handbook 10th edition (updated June 2015): Commonwealth of Australia 2015.

21. National Vaccine Storage Guidelines 2013 - Strive for Five. Australian Government Department of Health and Ageing, 2013.

22. Finn A, Morales-Aza B, Sikora P, Giles J, Lethem R, Marlais M, et al. Density Distribution of Pharyngeal Carriage of Meningococcus in Healthy Young Adults: New Approaches to Studying the Epidemiology of Colonization and Vaccine Indirect Effects. Pediatr Infect Dis J. 2016;35(10):1080-5. Epub 2016/05/27. doi: 10.1097/inf.0000000000001237. PubMed PMID: 27228196.

23. Claus H, Maiden MC, Wilson DJ, McCarthy ND, Jolley KA, Urwin R, et al. Genetic analysis of meningococci carried by children and young adults. J Infect Dis. 2005;191(8):1263-71. Epub 2005/03/19. doi: 10.1086/428590. PubMed PMID: 15776372.

24. Killip S, Mahfoud Z, Pearce K. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers. Ann Fam Med. 2004;2(3):204-8. Epub 2004/06/24. PubMed PMID: 15209195; PubMed Central PMCID: PMCPMC1466680.

25. Plikaytis BD, Stella M, Boccadifuoco G, DeTora LM, Agnusdei M, Santini L, et al. Interlaboratory Standardization of the Sandwich Enzyme-Linked Immunosorbent Assay Designed for MATS, a Rapid, Reproducible Method for Estimating the Strain Coverage of Investigational Vaccines. Clinical and Vaccine Immunology : CVI. 2012;19(10):1609-17. doi: 10.1128/CVI.00202-12. PubMed PMID: PMC3485892.